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CHEMICAL ECOLOGY AND MARINE CARNIVORY: THE ROLE OF FREE AMINO ACIDS AS NATURAL FEEDING ATTRACTANTS

by

John Everington Commins

Bachelor of Science
United States Air Force Academy, 1994

Submitted in Partial Fulfillment of the
Requirements for the Degree of Master of Science
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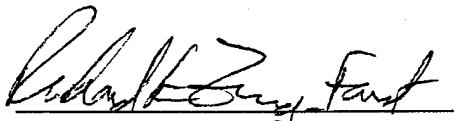

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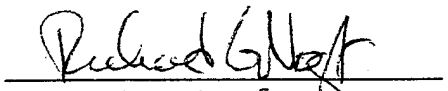

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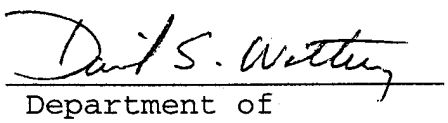

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ABSTRACT

The chemical senses of olfaction and taste are important determinants of animal feeding responses. Prior laboratory physiological and behavioral studies have demonstrated that compounds such as amino acids are potent feeding attractants for a variety animals. However, few investigators have explored the ecological interactions associated with amino acids and animal response in natural aquatic settings. Evidence from this study demonstrates the realized potential of amino acids to invoke carnivory of the mud snail, Ilyanassa obsoleta, in an estuarine tidal creek habitat. Results reveal that mixtures replicating the concentrations, compositions, and input rates of dissolved free amino acids released from fluids of a freshly killed fiddler crab, Uca pugilator, were able to induce mud snail feeding behavior and account for the full attractive capacity of the fiddler crab carrion. However, an amino acid mixture replicating the hard clam, Mercenaria mercenaria, was less attractive to mud snails than the corresponding freshly killed clam. Based on the ability of amino acids, in some cases, to invoke mud snail carnivory under natural conditions, a test of mixture composition and the importance of flux, the amount of chemical attractant released over time, was conducted. Mud snail attraction was found to be dependent upon the amount of total amino acids

present, not a specific mixture composition. Results also suggest that flux mediates mud snail feeding behavior, not fluid input rate or concentration, per se.

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Jason Whittle, and Mark Wiggins who provided assistance and moral support over the last year.

INTRODUCTION

Chemoreception mediates behavioral and ecological interactions that allow organisms to detect and find valuable resources (Daloze et al., 1980; Atema, 1985; Carr, 1987) In terrestrial environments, organisms are attracted to airborne volatiles like alcohols, aldehydes, esters, and aromatic hydrocarbons (Alm et al., 1985; Blum, 1988; Metcalf and Lampman, 1991; Phillips, 1993). By contrast, solubility rather than volatility is usually thought to determine the types of compounds principally serving as attractants to organisms living in aquatic habitats (see Laverack, 1974; Hara, 1992). Important similarities, however, can be found in the molecular identities of substances either stimulating or attracting both terrestrial and aquatic organisms. In particular, water soluble agents such as free amino acids, sugars, and nucleotides often promote both exploratory and consummatory phases of feeding in a vast majority of organisms regardless of habitat (Lindstedt, 1971; Takeda, 1980a,b; Mackie and Mitchell, 1982; Dadd and Kleinjan, 1985). Whereas terrestrial organisms respond only after contacting these water soluble compounds in fluids associated with particulate material, aquatic organisms often act before such contact due to chemical transport in flowing water.

The roles of amino acids as feeding stimulants and attractants have been especially well investigated (Carr, 1982; Carr, 1987; Hara, 1992; Lanza et al., 1993). These substances are not only building blocks and breakdown products of proteins, but they serve as osmolytes in maintaining cell volume (Zurburg and DeZwaan, 1981; Yancey et al., 1992). Concentrations of amino acids can occur at very high concentrations (1 - 100 mM) in a wide variety of sources, including the body fluids of animals (Hashimoto et al., 1968; Suyama and Suzuki, 1975; Carr, 1987) and the nectar of flowering plants (Inouye and Inouye, 1980; Gottsberger et al., 1984).

Amino acids have served as important substrates for investigations on chemoreceptive physiology and behavior of taxonomically diverse organisms, including bacteria (Boyd and Simon, 1982; Ordal, 1985), insects (Inouye and Waller, 1984; Lanza et al., 1993), crustaceans (Ache, 1982; Carr and Derby, 1986a), fish (Atema, 1980; Caprio and Byrd, 1984), and rats (Iwasaki et al., 1985; Grill and Flynn, 1987), among others. For example, tremendous progress has been made on the biophysics of chemosensory transduction events in isolated chemosensory receptor neurons using amino acids as ligands (Bruch and Kalinoski, 1987; Baxter and Morse, 1992; Lo et al., 1993). Furthermore, much has been learned about olfactory and taste discrimination by electrophysiological and behavioral investigations on cellular and on whole animal behavioral responses to single

amino acids and to complex mixtures comprised principally of amino acids (Shelton and Mackie, 1971; Carr, 1978; Derby and Atema, 1982, 1987; Zimmer-Faust et al., 1984; Carr and Derby, 1986a,b).

A major goal of investigators studying olfaction and taste has been to understand how organisms detect and perceive the quality and quantity of chemical stimuli. This knowledge is important to physiologists, but also to ecologists, because the choices organisms make in selecting resources often depend on such properties. It is now widely believed that chemical quality is determined by either recognizing substances expressing novel molecular structures (e.g. Mackie and Adron, 1978; Boeckh, 1980; Carr et al., 1986), or else discriminating a unique pattern in the way compounds lacking novel structures are blended together (Mackie, 1973; Ohsugi, Hidaka, and Ikeda, 1978; Carr, Netherton, and Milstead, 1984; Carr and Derby, 1986a,b). Recently, research using both associative, non-associative, and aversive conditioning paradigms have shown that Florida spiny lobsters (Panulirus argus) learn to distinguish between mixtures composed of common amino acids, nucleotides, organic acids and organic bases, held at the same overall concentration, but differing in percent composition (Fine-Levy et al., 1988, 1989; Derby et al., 1989; Daniel and Derby, 1990). By contrast, the ability to perceive chemical quantity is believed to arise from the concentration of stimulatory molecules. A large number of

previous investigations have clearly demonstrated that the electrophysiological responses of chemosensory receptor neurons, as well as whole-organismal behavioral reactions, usually increase linearly as stimulus concentration increases logarithmically (Mackie and Shelton, 1972; Fuzessery and Childress, 1975; Derby and Atema, 1982; Carr and Derby, 1986a; Derby and Atema; 1987). Chemoreceptive behavior measured either at cellular or at organismal levels is therefore concentration dependent.

Surprisingly, with the exception of terrestrial arthropods (e.g. Murlis et al., 1982; David et al., 1983; Elkinton et al., 1987; Hall, 1987; Willis et al., 1991), very few field studies have been performed on the ecology of chemical sensing. Current understanding of both quality and quantity coding of chemosensory information, particularly among aquatic organisms, has been established almost entirely from laboratory physiological and behavioral investigations (see Carr, 1987; Derby and Atema, 1987; Derby et al., 1989; Zimmer-Faust, 1989; Derby et al., 1991a,b). Because of the tremendous body of knowledge built from laboratory assays on their role as feeding attractants and stimulants, amino acids provide excellent tools for use in aquatic field investigations. Currently, we tested the ability of free amino acids to invoke carnivory among populations of mud snails, Ilyanassa obsoleta, inhabiting a tidal estuary. Using amino acid compositions, concentrations, and input rates simulating fluids naturally

released from freshly-killed fiddler crabs (Uca pugilator) and hard clams (Mercenaria mercenaria) carrions, we determined the effect of mixture blend on the attraction of snails to chemical release sites. Finally, we also established the relative importance of amino acid concentration and flux, the amount of chemical stimuli released over time, on mud snail chemoattractivity. Whereas laboratory physiological and behavioral investigations are critical in establishing the potential or scope for animal response, field trials are valuable in verifying when and where this potential is realized.

MATERIALS AND METHODS

Description of study site and chemical transport

All experiments were conducted from August to November 1994 in the North Inlet Estuary, near Georgetown, South Carolina. A permanent 50 m X 15 m band transect was established in a tidal creek near Oyster Landing and experiments were performed during low tide with water depths of 1 - 3 cm. Conductivity and water temperature were continuously recorded at 0.5 h intervals using a CTD probe (Datasonde 3, Hydrolab Corp.) mounted 18 cm above the creek bed at Oyster Landing, where water depth was never less than 0.3 m. Prior studies have well characterized this area in terms of vegetation, sedimentary, and geochemical features (see review by Blood and Vernberg, 1992). We currently established grain sizes of sediments sampled from areas where experiments were conducted. Each sediment sample (1 g dry weight; n = 45) was heated to 500 ^{o}C for 4 h to remove organics, then both lengths and widths of 300 individual grains were measured using an Olympus BH-2 epifluorescence compound microscope and ocular micrometer.

Water flow speed was measured at the end of each experimental trial by injecting a small patch of fluorescein dye and determining the time required for the center of the dye patch to travel 1 m downstream. We prepared the fluorescein (1 g l^{-1}) in seawater drawn from the study site,

then released this solution through polyethylene tubing (I.D. 1.14 mm, O.D. 1.57 mm). More detailed hydrodynamic measurements were periodically made at the study site and care was taken to ensure that we sampled the full range of flow conditions occurring during experiments. Both advection and turbulent mixing coefficients were measured by analyzing video records of fluorescein dye labeled plumes. In each of 15 trials, fluorescein was continuously introduced at 6 ml min^{-1} using a modified syringe pump (Sage Model 351, Orion Instruments) operated by a portable electrical generator. The plumes were recorded using a video camera (Sony TR81) mounted 2 m above the tidal creek with a scale bar placed in the field of view. From the videorecords we estimated turbulent mixing coefficients as rates of change in the across-stream variance in fluorescein concentration over time (Denny, 1988). Our previous study showed that estimates of mixing coefficients based on either fluorometric determinations or on flow meter records (using the eddy correlation method) were essentially identical (Zimmer-Faust et al., 1995).

When measured at fast temporal scales, chemical stimuli in plumes are patchily distributed due to turbulence. Mean concentrations and time-averaged distributions of fluorescein dye, therefore, may not be indicative of the information available to snails navigating towards the source of chemical release. To achieve faster time resolution in our characterization of chemical transport

dynamics, on 12 occasions, we employed carbon fiber microelectrodes (150 μm diam.) and a computer recording system (Model IVEC-10, MedSystems Corp.) to sample dopamine as a chemical tracer at 10 Hz. All methods used in making these recordings and justification for employing dopamine as a chemical tracer were previously described (Zimmer-Faust et al., 1995). Currently, we limited recordings to sites located along the plume midline (as visualized with fluorescein dye) at 5, 15, and 25 cm distances from the dopamine release site. The microelectrode sensor was always positioned within 1 mm of the creek bed to simulate the natural posturing of the snail siphon, which acts in pumping water over the osphradium (the olfactory organ).

Description of mud snail populations

Population densities were measured every month by counting snails in each of ten, 0.5 m^2 quadrats randomly placed in the permanent 50 m X 15 m study area. All individuals either exposed on surface sediments or buried above the black reducing layer were counted, and shell height (spire tip to aperture base) was measured for each snail. Gender and percentage parasitized were assessed each month by randomly sub-sampling 100 snails of the total counted. Another 100 snails that responded positively to chemical stimuli were collected each month, and size, gender and presence or absence of parasites determined. Males were identified based upon the presence or absence of a penis or

penis stub. Snails were considered parasitized if sporocysts or redia of trematodes were present in the gonads as described by Stunkard (1983).

Mud snail attraction to live prey, freshly killed carrion, and DFAAs

General procedures:

Mud snails are considered to be opportunistic facultative carnivores feeding upon carrion when available (Hurd, 1985). Both live hard clams and fiddler crabs are abundant and they commonly occur as carrion in the tidal creeks inhabited by mud snails (Commins and Zimmer-Faust, unpubl. data). We measured mud snail attraction to live and freshly killed fiddler crabs and clams, along with chemical mixtures simulating precisely the amino acid compositions and concentrations of fluids released by fiddler crab and clam carrions. To measure chemical attraction, we constructed a testing apparatus consisting of a 2.5 cm radius ring fastened to the inner portion and in the center of a larger 25 cm radius ring. Disturbance to the natural water flow was minimized by inserting four 50 cm long threaded rods (each 0.25 cm diam) through the 25 cm radius ring. Each rod was held in place by two threaded fasteners which enabled us to lower or raise the testing apparatus to a height of 10 cm above the surface of the water.

Before each trial, the testing ring was placed a population of mud snails in which the flow of the water over the test section was not impeded by any large scale topographical features, such as fecal mounds or depressions in the substratum. After the testing apparatus was in place, snails located within the inner 2.5 cm radius ring were removed prior to testing. Snails outside and within a 25 cm distance of the testing apparatus were also removed to prevent them from entering the ring during an experiment. Snails inside the 25 cm radius ring and outside of the 2.5 cm radius ring were counted, and the number recorded prior to any experiment (mean = 55 $\pm$ 0.5 SEM; range = 45-65). All chemical attractants were introduced in the center of the 2.5 cm radius ring. Once a trial had begun, any snails entering the 2.5 cm radius ring were counted as positively responding to the presented chemical stimuli. Finally, any snails that emerged buried in the sediment during a trial were removed and were not counted toward the testing apparatus population total or as positively responding to the chemical stimuli. All experimental trials lasted for 5 minutes, and at the end of each trial water depth at the center of the testing apparatus was recorded.

Mud snail attraction to live prey and freshly killed carrion:

Experiments measuring snail response to live and freshly killed fiddler crabs and clams were done in the following

way. Either a live (2.0 g wet tissue mass) fiddler crab or clam was secured inside a 2 cm x 3 cm vexar mesh bag (mesh size = 1 mm^2) and placed inside the center of the inner 2.5 cm radius ring of our testing apparatus as previously described. The bag was fixed firmly to the top of the substratum by a wire anchored into the sediment. For all trials in which live fiddler crabs were used as the source of chemical attraction, a trial began once the vexar mesh bag was placed in the middle of the 2.5 cm radius ring. Trials involving live clams did not start until the clam, secured to the substratum in a vexar mesh bag, their siphons were gaped and extended.

We prepared carrion by either using an thoroughly rinsed and dried autotomized blue crab claw to pierce a fiddler crab or chip a clam. Our previous investigation showed that the chemical compositions and fluid release rates from carrion used in these experiments and from carrion naturally attacked by blue crabs were essentially identical (Zimmer-Faust et al., in prep). The damaged clam or fiddler crab was then placed inside of a vexar mesh bag and secured to the substratum by a wire in the center of the 2.5 cm ring. Controls for both live and carrion experiments consisted of depositing an empty mesh bag within the middle of the testing apparatus.

Comparison of mud snail attraction to carrion and corresponding DFAA mixtures:

DFAA composition, concentration, and input rates from both live and freshly killed clams and fiddler crabs were previously determined (Tables 1 and 2; Zimmer-Faust et al., in prep.) In this study we prepared synthetic DFAA mixtures to mimic the compositions and concentrations of substances released from fresh clam and crab carrion. All DFAA solutions were prepared in artificial sea water (ASW, particles and organic free) and stored at -80° C until use in experiments. Both clam and fiddler crab DFAA solutions were introduced at rates equal to their amino acid input rates as previously determined (Table 2). Each solution was delivered through 18 gauge intramedic polyethylene tubing (I.D. 1.14 mm, O.D. 1.57 mm) by a syringe pump (Sage Model # 351). Controls for all experiments consisted of introducing ASW at the higher release rate, 0.12 ml min^{-1}, of amino acids from fiddler crab carrion. All trials lasted for 5 minutes.

Experiments ascertaining the importance amino acid composition:

Because the concentration of individual amino acids are different between the fiddler crab and clam, easily signified by taurine (55% of the total amino acids released by a clam versus 11% for a fiddler crab), an experiment was conducted to determine if amino acid composition plays a

role in the attraction of mud snails to carrion. We prepared a DFAA synthetic mixture based upon the amino acid composition released from a freshly killed fiddler crab; however, the total amino acid concentration and release rate were reduced to match that leaking from clam carrion. Similarly, a clam carrion DFAA solution was made by increasing the overall concentration of amino acids to that of a freshly killed fiddler crab, while holding the relative proportion of clam carrion individual amino acids constant. This solution was delivered at the same rate as released from fiddler crab carrion. Mud snail attraction was therefore measured using DFAA synthetic solutions whose input rate and concentration, or flux, were equal, but differed in amino acid composition. The ASW controls for this experiment were all introduced at a rate of 0.12 ml min^{-1}.

Experiments assessing the importance of amino acid flux:

Varying input rates and concentration: An experiment was conducted to determine the relative dependence of mud snail attraction on DFAA input rate, concentration, and flux. The flux of a fiddler crab DFAA solution was introduced at three levels (1.2, 2.3, 6.8 $\mu M\ min^{-1}$). Three different concentrations and input rates were used while the flux was held constant at each flux level. To achieve equal flux rates, one of the two solutions had a higher concentration of DFAAs, while the other solution was

introduced at a higher input rate. All ASW controls were introduced at 0.12 ml min^{-1}.

Flux/Response Relation: The relationship between DFAA flux and mud snail attraction was examined by obtaining a flux/response curve. The input rate of a DFAA synthetic mixture mimicking freshly killed fiddler crab carrion was maintained at 0.12 ml min^{-1} while the concentration was repeatedly diluted to give 5 discrete flux levels. Controls for this experiment were ASW solutions introduced at a rate of 0.12 ml min^{-1}.

Percentage of mud snails retested:

Because some experimental trials were repeated in the same area on consecutive days (minimum of five days between a two day testing period and the next testing date), we ascertained whether snails were being retested. Ten circular plots with a radius of 250 cm were randomly chosen in the tidal creek where experiments were conducted. Mud snails inside each plot were counted and marked with typewriter correction fluid. The location of a plot was noted by driving a 1 m stick deep into the center. The following day the total number of marked snails inside all of the ten 250 cm plots were counted and recorded to determine the percentage of snails that would have been retested if experiments were conducted over a two day period.

Statistical analysis:

For experiments investigating the attraction of mud snails to live or carrion fiddler crabs and clams, along with DFAA synthetic mixtures, one-way analysis of variance (ANOVA) was employed. A one-way ANOVA was also used to ascertain the role of amino acid composition on the attraction of mud snails. To determine the relation between mud snail response and the flux of an introduced DFAA synthetic mixture, linear regression and one-way ANOVA were utilized in which the flux rates were log transformed. For all one-way ANOVAs, selected factor level means were compared using a Bonferroni method of multiple comparisons. The percentage of mud snails in the testing apparatus attracted to chemical attractants for all experimental trials were arcsine transformed. A Fisher's exact test was used to determine differences in sex ratios and incidence of parasitism between mud snails positively responding to chemical attractants and those randomly sampled from snail populations. Finally, a two-sample t test was used to distinguish differences in shell aperture length between snails randomly chosen from populations and those positively responding to any introduced chemical stimuli.

RESULTS

Physical and Chemical Characteristics of Study Site

Salinity levels ranged between 29.1 o/oo and 35.1 o/oo, while the water temperature was between 17.7 ° C and 25.5° C. Flow speeds for all experimental trials averaged 15.9 cm s^{-1} ($\pm$ 1.2 cm s^{-1} SEM; N = 136 trials), and water depth averaged 1.8 cm ($\pm$ 0.1 cm SEM). Sediments were muddy clay with a mean grain size of 11 µm ($\pm$ 2 µm SEM).

Data obtained from measuring the concentration of a chemical tracer over time showed the flow environment to be highly variable. For each microelectrode recording (n = 6) made, the different flow regime, the average dopamine concentration decreased as the electrode was moved away from the input source. Figure 1 shows a recording at a flow speed (14 cm s^{-1}) and a depth (1 cm) typical of the average conditions maintained in our experiments. Significantly, the peak concentrations established even 5 cm away from the input source were diluted about 250 times from the source strength, i.e., from 20 mM to 80 µM, while the mean concentration was diluted 950 times, i.e., from 20 mM to 21 µM. Mean concentrations were further diluted to 10 µM and 2 µM at sites positioned 15 and 25 cm downstream of the input source. Turbulent mixing coefficients for all flow regimes ranged from 0.8 cm^2 s^{-1} to 2.1 cm^2 s^{-1}. Remarkably for every recording, the concentration of dopamine never reached

zero even at distances of 25 cm away from the input source. Perhaps, the electrode had penetrated the viscous sub-layer or else the depth limitation of water flowing in the tidal creek possibly limited vertical and horizontal dispersal and caused increased mixing through the shallow (1-3 cm) water column.

Characteristics of Mud Snail Population

Snail populations reached up to 645 individuals m^{-2} with a mean density of 145 individuals m^{-2}. The lowest mean shell aperture length of randomly chosen snails sampled during one month was 15.7 mm (± 0.11 SEM), n = 100, while the highest length of sampled snails was 16.2 mm (± 0.13 SEM), n = 100. Of all the snails dissected the highest incidence of parasitism was 13% while the lowest was 6%. The sex ratio ranged from a high of 2.4:1 to a low of 2:1, while the sex ratio for attracted snails ranged from 2.8:1 to 2.2. The percentage of attracted snails parasitized was between 8% and 4%, and the shell height ranged from, 15.5 mm (± 0.14 SEM), n = 100, to 15.8 mm (± 0.098 SEM), n = 100. No difference in sex ratio was found between the snails sampled from tidal creek populations and those that had responded positively to either live or carrion prey or to a DFAA synthetic mixture (Fisher's exact test: df = 1; p = 0.493). Parasitism and shell length of attracted snails also did not differ from those randomly chosen from snail populations (Fisher's exact test: df = 1; p = 0.473

[parasitism] and two-sample t statistic: $t = 1.305$ $df = 135$; $p < 0.05$ [shell length]).

The retesting rate for attractive mud snails was low during field experiments. Only 11% (1284 marked, 141 retested) of all the snails marked were found the following day inside any of the ten circular quadrates.

Attraction to live and freshly killed prey and DFAA solutions

Responses to intact live prey and freshly killed carrion:

Results indicate that mud snails were not attracted to live clams or fiddler crabs (Figures 2 and 3). Differences between live clams and fiddler crabs versus empty mesh bags were not significant when using a Bonferroni multiple pair-wise comparison test ($p = 0.2324$ and 0.4378 respectively, $n = 8$ for both comparisons). However, snails were significantly more attracted to cracked clams and pierced fiddler crabs than to the empty mesh bags (Bonferroni test: $p < 0.0001$ and $n = 8$ for both comparisons). These findings suggest that the release of natural stimulants by live intact prey may not be capable of attracting snails, but that the stimulus released from a pierced fiddler crab or cracked clam is capable of inducing snails to seek out injured prey. The percentage of snails responding to clam and fiddler crab carrion did not significantly differ (Bonferroni test: $p = 0.1547$, $n = 8$). Therefore, carrion

type did not seem to be a factor in mediating snail response to a potential food source.

Responses to DFAA artificial solutions mimicking carrion prey:

Attractions of mud snails to the clam carrion DFAA synthetic mixture mimicking the amino acid composition, concentration, and release rate of a freshly killed clam were significantly greater than the ASW control (Figure 2) (Bonferroni test: $p = 0.0011$, $n = 8$). However, a significantly greater proportion of mud snail responded to the clam carrion than to the DFAA artificial mixture (Figure 2) (Bonferroni test: $p < 0.0001$, $n = 8$). Responses of mud snails to fiddler crab carrion DFAA synthetic mixtures were equivalent to its carrion counterpart (Figure 3) (Bonferroni test: $p = 0.5039$, $n = 8$). These results imply that attraction of mud snails to DFAAs depends upon the animal type, which dictates flux of amino acids from prey fluids. The difference in responses to clam carrion and its DFAA synthetic mixture complement indicates that mud snail attraction to clam carrion is not entirely due to the presence of amino acids; that other sources of attraction must be present in the body fluids released from a cracked clam.

Role of amino acid composition:

The relative make-up of amino acids does not play a significant part in the attraction of mud snails to DFAA synthetic mixtures (Figure 4). However, results also reveal that there was no significant difference between the proportion of snails responding to solutions at clam carrion fluxes, but possessing an amino acid compositions of either a fiddler crab or clam (Bonferroni test: $p = 0.9449$, $n = 5$). Likewise, there was no difference between the amount of snails responding to DFAA solutions possessing either a fiddler crab and clam amino acid compositions, but introduced at a fiddler crab flux (Bonferroni test: $p = 0.5473$, $n = 5$). All DFAA synthetic mixtures were however, significantly different from the ASW controls (Bonferroni test: $p < 0.0001$ and $n = 5$ for all comparisons).

Importance of Flux:

The relative importance of flux was examined by comparing responses of mud snails to DFAA synthetic solutions at three flux levels. Comparisons were made between two solutions at each flux level in which one solution contained a higher concentration while the other solution was introduced at a faster rate. The responses to the two solutions for each flux level did not differ significantly (Table 3). In addition, as flux increased so did the proportion of snails responding to a DFAA synthetic mixture ($r^2 = 0.79$, $p < 0.0001$, $n = 50$) (Figure 5). Results

demonstrate that the overall flux of a chemical attractant determines the chemical response of mud snails.

Finally, when I compared mud snail response over a range of flux levels to attraction to ASW controls, I found that there was no significant difference in snail response to a flux released at 0.28 μmoles min^{-1} and the ASW control (Bonferroni test: $p = 0.0495$). Responses to all other fluxes were significantly greater than the ASW control (Bonferroni test: $p \leq 0.0$, all comparisons). Thus, the threshold detectable flux must be between 0.28 μmoles min^{-1} and the next highest flux level of 0.57 μmoles min^{-1}.

DISCUSSION

Chemosensory responses of mud snails to amino acids were tested in a tidal creek habitat. Results indicate that amino acids released at natural carrion concentrations, compositions, and fluxes may act as chemical attractants. However, this may not always true as mud snails were not attracted to DFAA mixtures simulating a freshly killed clam. Thus, animals are only selectively attracted to amino acids. Input fluxes from carrion sources must be high for animals to perceive a sufficient chemical quantity. Mud snail attraction to amino acids released at a flux of 0.28 μmoles min^{-1} did not differ from ASW controls. Estimating the minimum detectable flux capable of invoking mud snail carnivory to be no lower than this flux, it is reasonable to assume that amino acids released from live fiddler crabs and clams do not attract mud snails. Natural fluxes of amino acids from these prey items are approximately 1000 times lower the estimated threshold flux. This may explain why mud snails are not attracted to live prey.

Remarkably, the role of amino acids delivered at rates equaling freshly killed prey has never been thoroughly quantified within natural aquatic conditions. In many investigations, attraction to DFAA solutions did not differ from controls (Ache et al., 1978; Zimmer-Faust and Case, 1983; Heatwole et al., 1988; Finelli et al., in prep).

Allen et al. (1975) occasionally obtained higher captures rates with glycine baited traps than with unbaited traps, but attraction to natural baits was never approached. DFAA mixtures used by Sutterlin (1975) and Mackie et al. (1980) attracted a greater number of animals than controls, but the flux of attractants was much higher than that released by naturally occurring carrion. In addition, capture rates of all natural and artificial baited traps by Mackie et al. (1980) were very low. Consequently, this current field study is the first to conclusively demonstrate the ability of free amino acids to mediate chemoreception of an aquatic animal under natural conditions.

The stimulatory and attractive capacity of amino acids has been identified by many laboratory behavioral and physiological studies from tests on an assortment of different organisms in a variety of aquatic habitats (see Lindstedt, 1971; Carr, 1982; Mackie and Mitchell, 1982; Carr, 1987; Derby and Atema, 1987; Marui and Caprio, 1992). Chemical stimulants are usually defined as substances promoting ingestion and continuation of feeding, while attractants are compounds that allow animals to detect and locate food sources over a distance (Dethier et al., 1960; Beck, 1965; Lindstedt, 1971). Surprisingly, prior laboratory investigations using mud snails demonstrated amino acids only played a minor role, if any, as feeding stimulants (Carr, 1967b; Gurin and Carr, 1971; Carr et al., 1974). The aim of these investigators were to discover

chemical compounds that were stimulatory, not attractive. The number of mud snail proboscis extensions was used as a measure of feeding behavior, not location of chemical stimuli from a measured distance. Hence, contradictions in findings from these experiments with my own may be the product of different testing procedures or the result of different compounds responsible for stimulating mud snail feeding.

However, contrasting results may simply be due to the different environments associated with laboratories and natural habitats. For instance, in each laboratory chemosensory experiment using mud snails, animals were tested in still water assays inside small petri dishes, permitting investigators easy access to observe and quantify mud snail proboscis extension (Carr, 1967a,b; Gurin and Carr, 1971; Carr and Gurin, 1974). This design increases experimental speed and ease and allows greater investigator control over environmental variables (Sutterlin, 1975; Daniel and Bayer; 1987; Zimmer-Faust, 1989). However, the consequences of such constrained non-flow environments, when measuring feeding stimulation or attraction for any aquatic animal, are that animal locomotory space is restricted (Zimmer-Faust, submitted), unnatural chemical stimulus patterns are produced (Sutterlin, 1975; Zimmer-Faust and Case, 1982; Zimmer-Faust, 1989) and the impact of water flow as a possible physical cue is ignored (Hodgson and Mathewson, 1971; Bell and Tobin, 1982; Brown and Rittschof,

1984; Baker, 1986; Weissburg and Zimmer-Faust, 1993, 1994). Nevertheless, such disparities with my current results demonstrate the need for combining laboratory and field investigations to fully elucidate the mechanisms regulating chemosensory mediated behavioral responses. Whereas the laboratory is valuable in isolating factors, fields studies are necessary to fully understand the ecological role played by chemoreception in mediating foraging and feeding and to serve as a final test.

Chemical quantity is assumed to be a significant cue determining animal feeding response (Zimmer-Faust and Case, 1983; Derby and Atema, 1987). My study demonstrates that the concentration of amino acids released from freshly killed fiddler crabs and clams does determine mud snail response. However, mud snail attraction is not solely dependent upon concentration. Results also indicate that by varying the input rate of DFAA solutions, there is a corresponding fluctuation in mud snail attraction. Consequently, it is the product of fluid input rate and concentration, hence flux, that governs chemosensory mediated behavior in the mud snail. The importance of flux, controlling animal navigation towards and subsequent location of a prey item is not unique in studies on the ecology of chemoreception. Previous terrestrial studies have examined pheromone plumes using both time-average Gaussian dispersion models (Bossert and Wilson, 1963; Elkinton et al. 1984; Stanley et al. 1985) and instantaneous

fine-scale models (Mikstad and Kittredge, 1979; Murlis and Jones, 1981; Murlis et al., 1992). For both of these models the flux of chemical attractants determines size and shape of the plume active space.

The role of flux becomes even more important when investigating chemical attractant release from natural carrion. Whether in terrestrial or aquatic habitats, the ability to replicate natural odor production is critical to our understanding of how physical processes affect both chemical transport and animal behavior. Past investigations in aquatic habitats have primarily focused on behavioral responses to chemical concentration (e.g. Shelton and Mackie, 1975; Sutterlin, 1975; Pearson et al., 1979; Zimmer-Faust, 1984; Ellingsen and Doving, 1986). As a result, chemical stimuli were introduced at unnatural fluxes; thus, it was difficult to determine the ability of the stimuli to produce chemosensory responses within a natural context from these studies.

Results from my experiments also suggest that mixtures of identical concentrations, but different amino acid compositions are equally effective as attractants to mud snails. Although the synthetic mixtures I presented to mud snails were made up from two biologically relevant amino acid compositions, these solutions differed considerably. Given the foraging strategy of mud snails as opportunistic facultative scavengers consuming a variety of prey items my findings come as no surprise (Dimon 1905). Dietary and

osmolyte requirements of marine organisms dictate which amino acids are available and abundant in carrion tissue (Clark, 1968; Gérard and Gilles, 1972; Clark and Zounes, 1977; Bowlus and Somero, 1979; Zurburg and DeZwaan, 1981; Yancey et al., 1982). These same amino acids diffuse at high rates from prey animals specifying living or freshly killed prey (Rittschof, 1980; Zimmer-Faust and Case, 1982). Thus, from an evolutionary standpoint it would seem likely that the tuning characteristics of mud snail chemoreceptors are coordinated to respond to amino acid signatures indicative of natural prey items. More tests are needed to identify to what degree chemoreceptors are broadly tuned to these amino acids.

Chemosensory discrimination of food sources has been demonstrated in a variety of animals: insects (Thorpe and Jones, 1937), fish (Atema et al., 1980), snakes (Fuchs and Burghardt, 1971; Arnold, 1978), starfish (Castilla, 1972), crustaceans (Derby and Atema, 1980; Zimmer-Faust, 1982) among others. However, the attractants involved in such discrimination have yet to be identified. Working with the spiny lobster, Panulirus argus, Fine-Levy et al. (1988, 1989) and Daniel and Derby (1990) found that when trained using differential aversive and nonassociative conditioning techniques, lobsters can learn to distinguish among different mixtures equal in concentration and comprised principally of amino acids. Unfortunately, it is unknown if these mixtures enable lobsters to distinguish among chemical

signals emanating from different prey items within a natural context. It may well be that some animals have the ability to discriminate between prey items by distinguishing novel features of chemical blends. However, it may also be true that such compositions will evoke a strong and equivalent response, regardless of how the blends might smell as indicated by my results.

Data obtained by sampling dopamine at fast rates reveals that amino acids are likely limited in their ability to invoke mud snail carnivory. The degree of turbulent mixing in tidal creeks inhabited by mud snails indicates that the chemical signal of dopamine is rapidly diluted in a relatively short time (Mackie, 1975; Ogura, 1975). My fast sampling of dopamine over time at a distance 25 cm downstream from the input source indicates that the mean concentration was reduced 10,000 fold from its original quantity. The measured level of dopamine 25 cm away was slightly higher than amino acid background levels (10^{-7}-10^{-8} M) (Mopper and Lindroth, 1982; Carr, 1987) where mud snail attraction still occurred. However, at even farther distances (1 m) the amount of dopamine was less than the theoretical detection limit of the system (10^{-9} M) and well below amino acid background levels (Commins and Zimmer-Faust, person. obs.). Coincidentally, amino acids did not attract mud snails from distances greater than 0.5 m (Commins and Zimmer-Faust, person. obs). If mud snails had not been so close to the source of amino acid input, it is

unlikely that they would have responded to the amino acid mixtures at all. Furthermore, samples of primary amines collected 3 m away from abalone muscle by Zimmer-Faust and Case (1982) showed that amino acids were only present over the first three hours after abalone muscle was placed in traps. However, capture of animals took place 7-10 hours from the initial entry of bait into the traps. A dual problem therefore exists for amino acids as long distance foraging cues; not only are they rapidly diluted in aqueous environments, but the quantity available from carrion sources dramatically decreases over time. Future studies are essential to establish or reject the axiom that amino acids are limited as long distance chemical signal in aquatic animals.

Results from this study provide evidence that amino acids delivered at natural concentrations, compositions, and input rates may at times act as chemical cues used by mud snails to locate food items. Mud snail attraction was not dependent on either a fiddler crab or clam amino acid mixture blend, but rather on the total amount of amino acids released from bodily fluids. Finally, neither concentration nor input rate solely dictate mud snail attraction; instead they work in a concerted manner such that flux actually determines animal chemoattraction. Additional chemosensory investigations are needed to further explore when and where amino acids and other possible attractants might mediate chemoreception in real-life setting. By identifying natural

feeding attractants, investigators can then focus on developing and testing theories, on how such attractants might mediate feeding behavior in natural habitats.

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Table 1. Total dissolved free amino acids (measured as nmoles g^{-1} min^{-1}) released by live animals and freshly killed fiddler crabs (Uca pugilator) and hard clams (Mercenaria mercenaria).

Condition	Hard clam	Fiddler crab
Live prey	0.5	0.4
Fresh carrion	88	6,804

Table 2. Concentration and %-compositions of dissolved free amino acids in fluids released from freshly killed animals fiddler crabs (Uca pugilator) and hard clams (Mercenaria mercenaria).

Amino acid	Fiddler crab		Clam	
	mM	%-composition	mM	%-composition
Glycine	14.0	12.4	0.413	18.6
Serine	12.6	11.1	0.057	2.6
Taurine	12.0	10.6	1.223	55.1
Alanine	11.1	9.8	0.146	6.6
Lysine	9.6	8.5	0.027	1.2
Arginine	8.4	7.4	0.043	1.9
Leucine	7.7	6.8	0.007	0.3
Glutamatic acid	6.9	6.1	0.136	6.1
Aspartic acid	5.1	4.5	0.977	4.4
Proline	4.4	3.9	0.017	0.8
Threonine	3.6	3.2	0.008	0.4
Valine	3.7	3.2	0.009	0.4
Isoleucine	3.7	3.2	0.007	0.3
Phenylalanine	3.4	3.0	0.006	0.3
Tyrosine	2.9	2.6	0.011	0.5
Methionine	2.5	2.2	0.007	0.3
Histidine	1.8	1.6	0.005	0.2
Total amino acid	113.4		2.220	
Ammonium	7.9		0.12	
Fluid input rate	0.12 ml min^{-1}		0.08 ml min^{-1}	

Table 3. Proportions of mud snail populations attracted to sites of dissolved free amino acid release. Asterisks (*) indicate that the difference between proportions of snail populations attracted to each paired treatment is non-significant (Bonferroni test: $P \geq 0.37$, $n = 5$, all comparisons)

Flux (μmoles min^{-1})	Input rate (ml min^{-1})	Concentration (mM)	Proportion responding ($x \pm SEM$)
13.6	0.12	113.4	0.44 ± 0.02
6.8	0.12	56.8	0.29 ± 0.02*
	0.06	113.4	0.33 ± 0.02*
2.3	0.12	18.9	0.25 ± 0.02*
	0.02	113.4	0.27 ± 0.04*
1.2	0.12	9.5	0.21 ± 0.03*
	0.01	113.4	0.21 ± 0.03*

Figure 1. Profiles of dopamine (tracer) concentration sampled continuously at 10 Hz over 60-s intervals. Dopamine concentrations were measured at the plume midline (X = 0 cm) either (A.) 5 cm, (B.) 15 cm, or (C.) 25 cm downstream. For comparison, a dopamine profile was recorded (D.) outside of the plume as a control to evaluate background.

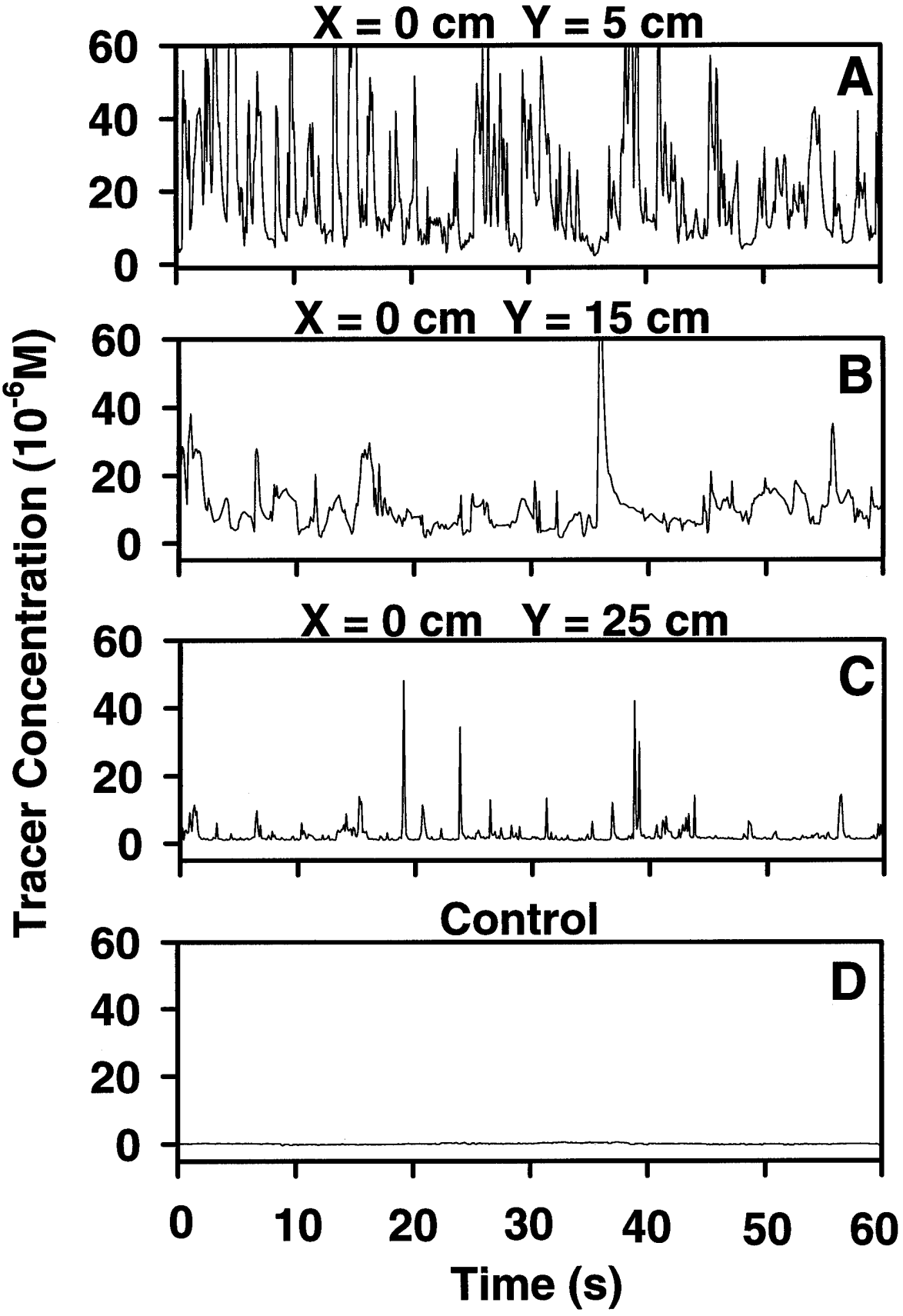

X = 0 cm Y = 5 cm
A
X = 0 cm Y = 15 cm
B
X = 0 cm Y = 25 cm
C
Control
D
60
40
20
0
Tracer Concentration (10-6M)
0
10
20
30
40
50
60
Time (s)

Figure 2. Proportion of mud snails attracted to mesh (1 mm^2) bags (2 cm x 3 cm) containing either a live or freshly killed (A.) fiddler crab or (B.) clam. Controls consisted of depositing an empty mesh bag into the center of the testing apparatus. Mud snail response was also assayed for DFAA synthetic mixtures replicating amino acid compositions, concentrations, and input rates released from fluids of freshly killed (A.) fiddler crabs or (B.) hard clams. ASW controls were introduced at the higher input rate, 0.12 ml min^{-1}, of amino acids released from fiddler crab carrion. Values are mean (± SEM) responses of mud snails located inside our 25 cm radius testing apparatus and subsequently entering the inner 2.5 cm radius ring where stimulus was introduced. Eight replicate trials were performed for each treatment

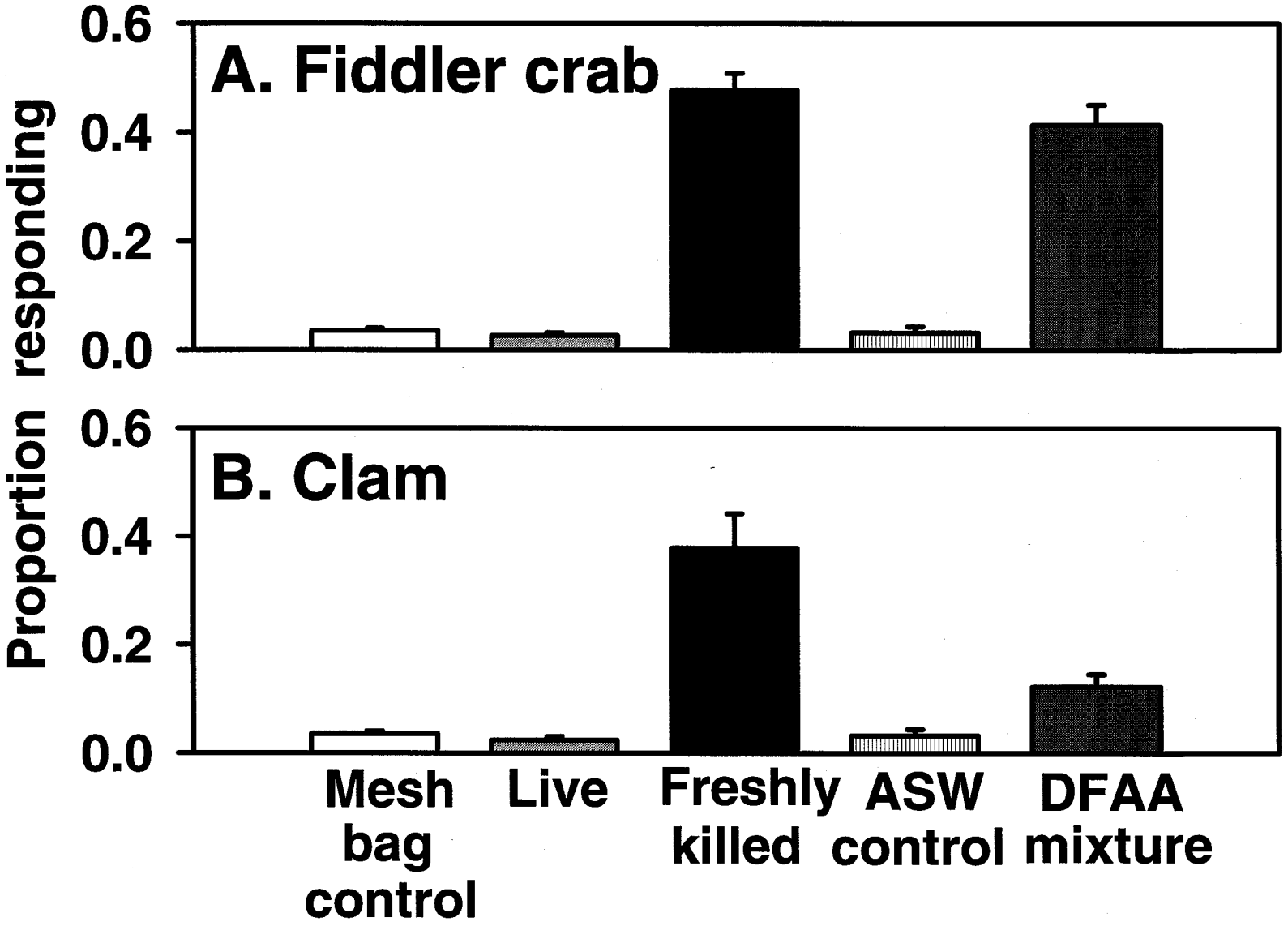

A. Fiddler crab
B. Clam
Proportion responding
0.6
0.4
0.2
0.0
Mesh bag control
Live
Freshly killed
ASW control
DFAA mixture

Figure 3. Proportion of mud snails attracted during 5-min exposures to DFAA synthetic mixtures whose input rate and concentration, or flux, were equal, but contained different, fiddler crab or hard clam, amino acid compositions. Controls (ASW) were introduced at the input release rate, 0.12 ml min^{-1}, of amino acids released from freshly killed fiddler crab carrion. Values are mean ($\pm$ SEM) responses of mud snails located inside our 25 cm radius testing apparatus and subsequently entering the inner 2.5 cm radius ring where stimulus was introduced. Eight replicate trials were performed for each treatment.

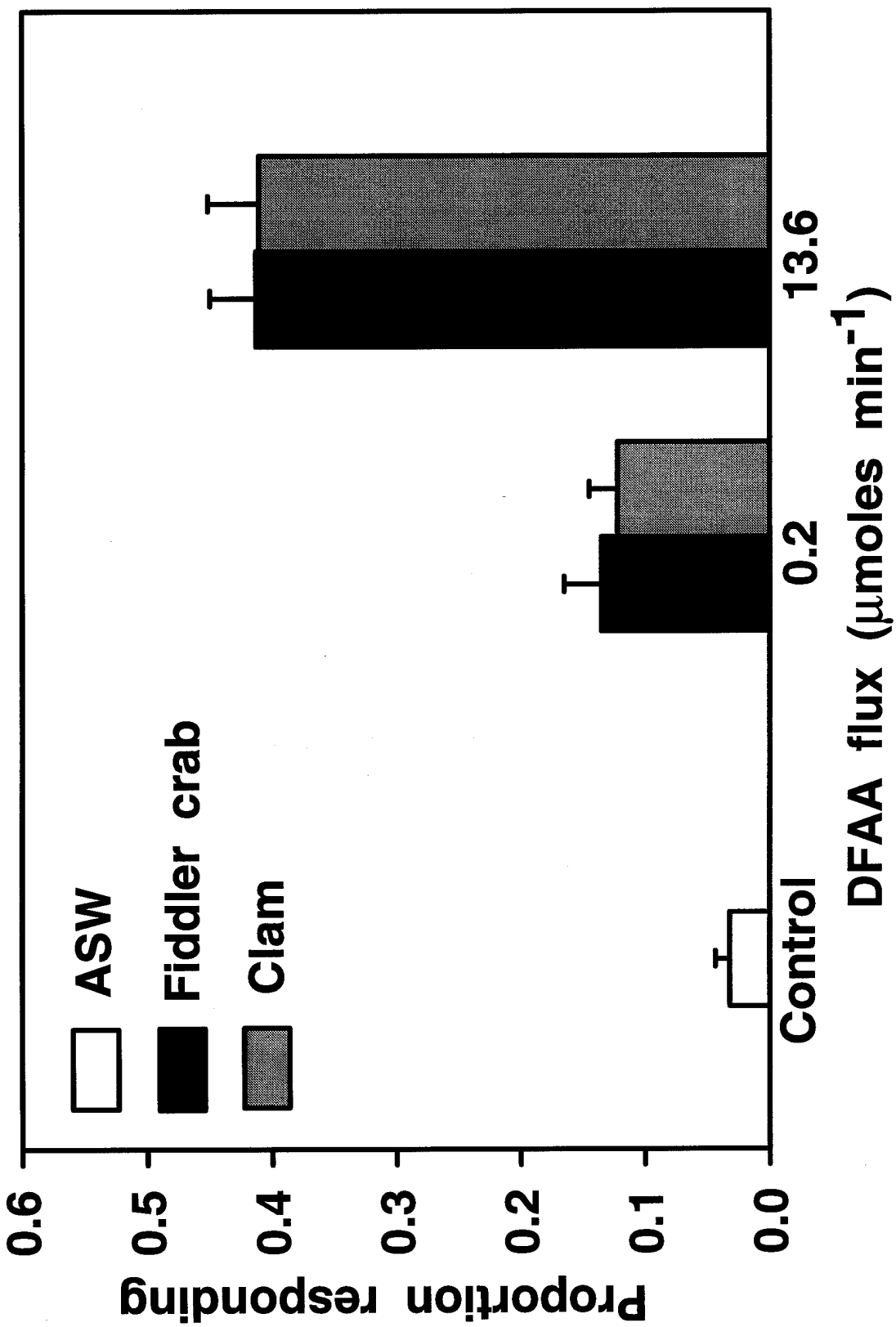
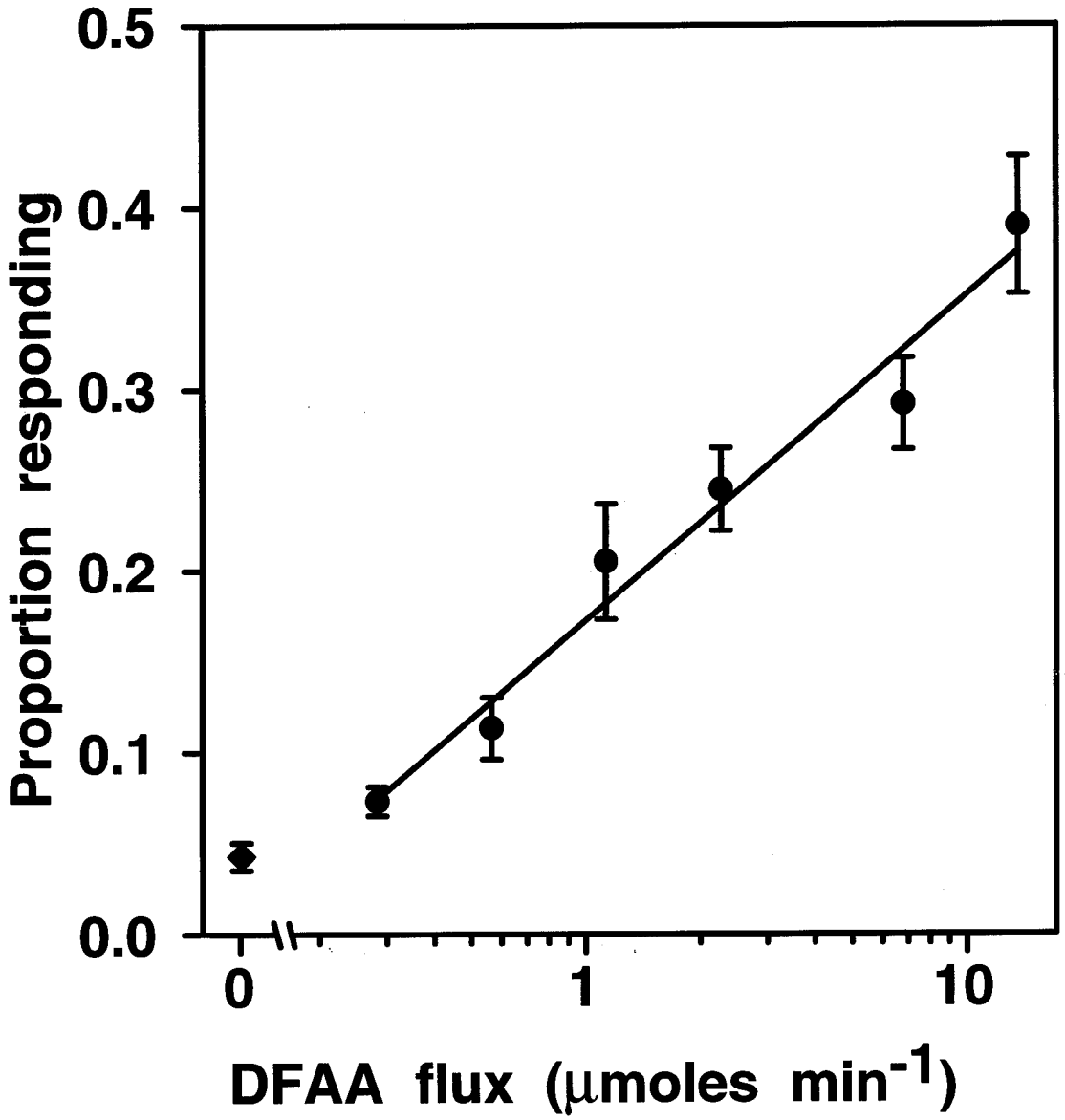

Proportion responding
0.0
0.1
0.2
0.3
0.4
0.5
0.6
ASW
Fiddler crab
Clam
Control
0.2
13.6
DFAA flux (μmoles min-1)

Figure. 4 Proportion of mud snails responding to DFAA synthetic mixtures, mimicking freshly killed fiddler crab carrion, over a range of flux levels. Values are mean ($\pm$ SEM) responses of mud snails located inside our 25 cm radius testing apparatus and subsequently entering the inner 2.5 cm radius ring where stimulus was introduced. Five replicate trials were performed for each treatment. ASW controls were introduced at 0.12 ml min^{-1}.


0.5
0.4
0.3
0.2
0.1
0.0
Proportion responding
0
1
10
DFAA flux (μmoles min-1)